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Amassing of Hydroxymethylfurfural, 2- Furfural and 5- Methyl furfural in orange (*Citrus reticulata*) juice during storage

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Abstract

Vitamin C is imperative component of our nutrition and used as additive in many foods owing to its high antioxidant activity. The degradation of vitamin C content in orange juice during storage was evaluated in this study. Degradation of vitamin C in orange juice during 40 days storage at 0, 05, 15, 25, and 40 °C was analyzed and evaluated. Hydroxymethylfurfural (HMF) accumulation in orange juice acts as an indicator of ascorbic acid degradation. The initial vitamin C content in orange juice was 34.26 mg/100 mL that reduced when subjected to various storage conditions such as temperature and time. The loss of vitamin C during storage was lowest at 0 °C and the losses increased with rise in temperature. The Vitamin C content after 40 days in orange juice at 0 °C were 7.19 mg/100 mL while the Vitamin C content reduced to 1.89 mg/100 mL in orange juice that were kept at 40 °C. Hence, with increase in temperature, the Vitamin C was degraded and accretion of HMF, 5 methyl furfural, and 2 methyl furfural was increased. The zero-order interaction between storage time and treatment showed significant influence on HMF.

Keywords: hydroxymethylfurfural; furfural; orange juice; storage; vitamin C degradation.

Practical Application: In the current study accumulation of furfural and its compounds in orange juice was determined which are the indicator of dilapidation of Vitamin C in juice during storage.

1 Introduction

Nutritional quality of food during storage is very important (Burdurlu et al., 2006) because most of nutritional loss take place during storage. Vitamin C (ascorbic acid) is present in appreciable amount in fruit juices but unfortunately, most of content of vitamin C degrade during storage and the rates of loss depends upon different storage conditions (Kabasakalis et al., 2000). Vitamin C is water soluble vitamin and its deficiency leads to a disease known as scurvy which is characterized by bruising and spontaneous haemorrhages under the skin, failure of wounds to heal, soft swollen gums, bleeding of the gums, and muscle fatigue (Maxfield & Crane, 2019).

Orange juice is an incredibly excellent source of ascorbic acid and its consumption in world is increasing at a tremendous rate. Ascorbic acid (AA) or vitamin C is an imperative component of human nutrition and also used as additive in many foods owing to its high antioxidant activity (Burdurlu et al., 2006; Del Caro et al., 2004). As an antioxidant, it acts as a free radical scavenger for preventing damage to the cell through by-products of chemical-cell interaction. The recommended daily allowance (RDA) for ascorbic acid is 100-120 mg/day which can reduce the risk of numerous maladies i.e. cancer, diabetes, cardiovascular, and neurological disorders. Vitamin C content in orange juice ranged from 150-450 mg/L and one glass of orange juice (200 mL) can deliver about 30-80% of RDA.

Ascorbic acid is an unstable compound and it is adversely affected by time and temperature (Klimczak et al., 2007). The content of vitamin C in different juices decreases during storage, depending on storage conditions such as temperature, oxygen, and amount of light exposed (Kabasakalis et al., 2000). Numerous decomposition reactive products like hydroxymethylfurfural (HMF), 2-furfural, and 5-methyl furfural are formed during the degradation of vitamin C. Both furfurals and HMF are often used as a measure of quality deterioration in citrus juices. The degradation products of ascorbic acid do not possess vitamin C activity. Several studies reported that furfural is one of the main degradation products of ascorbic acid or dehydro-ascorbic acid (Burdurlu et al., 2006). Storage of apple concentrates for 9 months at 25 °C resulted in HMF formation up to 27.9 mg/L (Spanos et al., 1990).

The existing research carried out on ascorbic acid degradation and resulting compounds is scant. Also, there is insufficient data regarding the quantitative assessment of degraded compounds of vitamin C. Therefore, keeping in view the above facts a project has been designed to evaluate the effect of storage duration

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as well as temperature on the degradation of ascorbic acid in orange juice. The other objective was to determine the quantity of hydroxymethylfurfural (HMF), 2-furfural, and 5-methyl furfural during storage.

2 Materials and methods

Fresh oranges were procured from local market. The oranges were kept at refrigeration temperature (4 °C) before processing into juice.

2.1 Processing of orange juice

Oranges were washed in tap water and cut into halves without peeling. Juice was extracted from oranges by using a rose head machine. The extraction rate of orange juice was 43%. The extracted juice was heated at 80 °C for one minute to inactivate enzymes. Following the heating process, the juice was rapidly cooled to room temperature and filtered through 8-fold cheese cloth to eliminate granular matter.

2.2 Storage of orange juice

The orange juice was filled in pre-sterilized glass jars, sealed and kept in darkness at 0, 5, 15, 25, and 40 °C for storage period of 40 days. The juice samples were analyzed for the presence of degraded products of ascorbic acid like HMF, 2-furfural, and 5-methyl furfural at an interval of 10 days for duration of 40 days.

2.3 Determination of ascorbic acid

Ascorbic acid was determined by using high performance liquid chromatography (HPLC) (Akalin et al., 2002) as given below. 7 mL of juice was added to 40 mL of buffer-acetonitrile mobile phase (0.5% (wt/vol) $(NH_4)_2HPO_4$ (0.038 M) 0.4% (vol/vol) acetonitrile (0.049 M), at pH 2.24 with H_3PO_4), extracted for 1 hour in orbital shaker (model 75, Burrell Scientific, Pottsburgh, PA), and centrifuged at 6000 x g for 5 minutes. The supernatant was collected and filtered once through Whatman No. 1 filter paper and twice through a 0.45-µm membrane filter (Satorious SM 11606, Goettingen, Germany) and then used directly for HPLC analysis. Triplicate analyses were performed on all samples. Analysis of ascorbic acid was made by HPLC with UV detector (Perkin Elmer-series 200) at 214 nm using RP-18 column (25 cm × 4.6 mm id). Quantification was based on the external standard method for accuracy while the Standard chromatogram is depicted in Figure 1.

2.4 Determination of furfuraldeydes

HMF, 2-Furfural, and 5-Methyl furfural were also determined using high performance liquid chromatography (HPLC) (Gökmen & Acar, 1999) as detailed below.

A 5 mL volume of orange juice was extracted twice, using a vortex mixer, with 10 mL of ethyl acetate by shaking vigorously for 1 min. The organic phases were combined and extracted with 2 mL of 1.5% sodium carbonate solution along with shaking for 1 min. The phases were allowed to separate and the aqueous phase was immediately extracted with 5 mL of ethyl acetate by shaking for 1 min. The combined organic phases to a total volume of 25 mL were dried over 2.5 g of anhydrous sodium sulfate. Subsequently, the dried extract was filtered through Whatman No. 1 filter paper to remove the remaining particles of anhydrous sodium sulfate. A 2 mL excess of ethyl acetate was added to wash the filter cake layer and the filtrate obtained was combined with the filtered extract. Then, the extract was evaporated just to dryness in a water bath at 40 °C under a gentle stream of nitrogen. The residue was immediately re-dissolved in 500 µL of HPLC water (pH 4.0) and 20 µL of this solution was

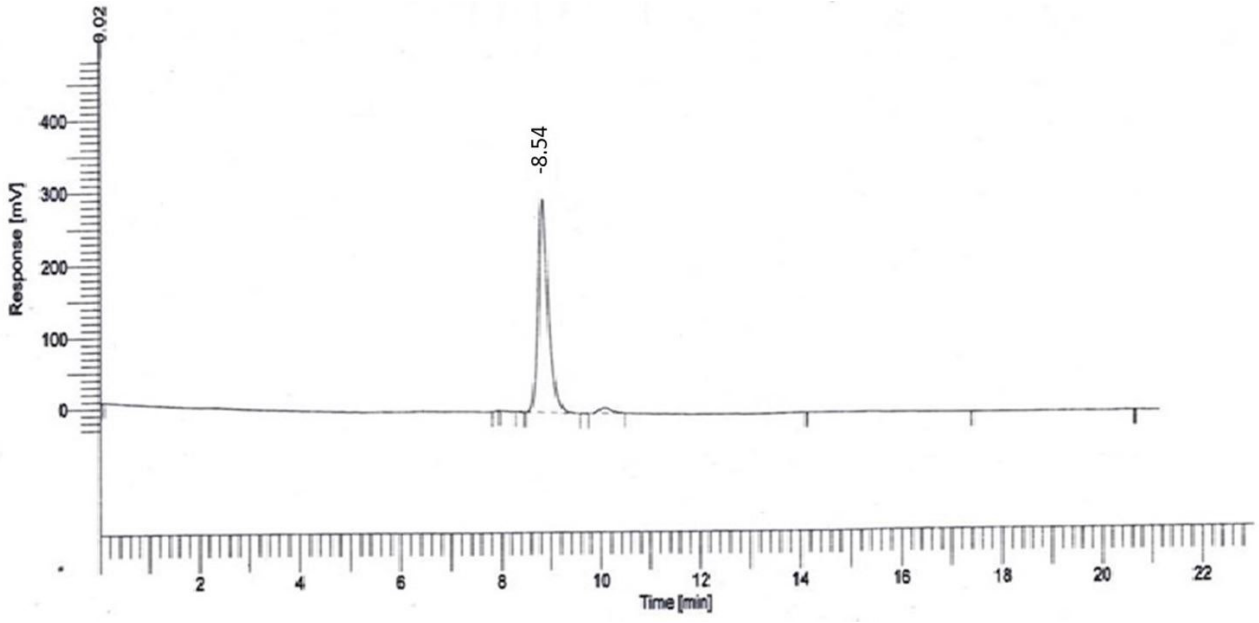

Figure 1. Standard Chromatogram of Ascorbic Acid.

injected into the column. The final solutions were kept frozen (-18 °C) until the chromatographic measurements.

Stock solutions of hydroxymethylfurfural, 5-methyl furfural, and 2-furfural were prepared by dissolving 1 g of each standard in 100 mL of ethyl acetate, then diluting these solutions 1:50 (v/v) by ethyl acetate to obtain a final concentration of 0.2 mg/mL for each compound. A 100 mL volume (each standard) of these stock solutions was transferred into 10 mL volumetric flasks and evaporated just to dryness under a gentle stream of nitrogen at room temperature. The residues were immediately dissolved in 10 mL of water (pH 4.0) acidified with acetic acid. Working standard solutions were prepared by appropriate dilution of these solutions with water (pH 4.0).

Quantification was done based on external standard method while the Standard chromatogram is depicted in Figure 2.

2.5 Statistical analysis

The data was imperiled to statistical analysis to determine the level of significance using 2-factor factorial CRD following the principles outlined by Steel et al. (1997).

3 Results and discussion

3.1 Loss of vitamin C during storage

Losses of vitamin C have been depicted in Table 1. Results showed that initial content of ascorbic acid in orange juice was 34.27 mg/100 mL. After 40 days of storage at 0, 5, 15, 25, and 40 °C the ascorbic acid content of samples decreased to 7.19, 3.04, 2.33, 2.22, and 1.89 mg/100 mL respectively.

It was observed during the experiment that destruction of vitamin C was much faster at the start of storage and after 10 days of storage at 0, 5, 15, 25, and 40 °C the ascorbic acid content of samples decreased drastically by 65.94, 86.81, 83.39, 90.11, and 91.10%, respectively but afterwards the decrease in vitamin C content was rather slow.

At the end of storage, the loss of vitamin C content was 79.01, 91.13, 93.20, 93.52, and 94.48% respectively at different storage temperatures mentioned above as compared to the start of the study. Ascorbic acid loss was more pronounced at 40 °C and it showed maximum decrease in ascorbic acid content throughout the storage period. The study showed that the decrease in the ascorbic acid content during storage was directly proportional to

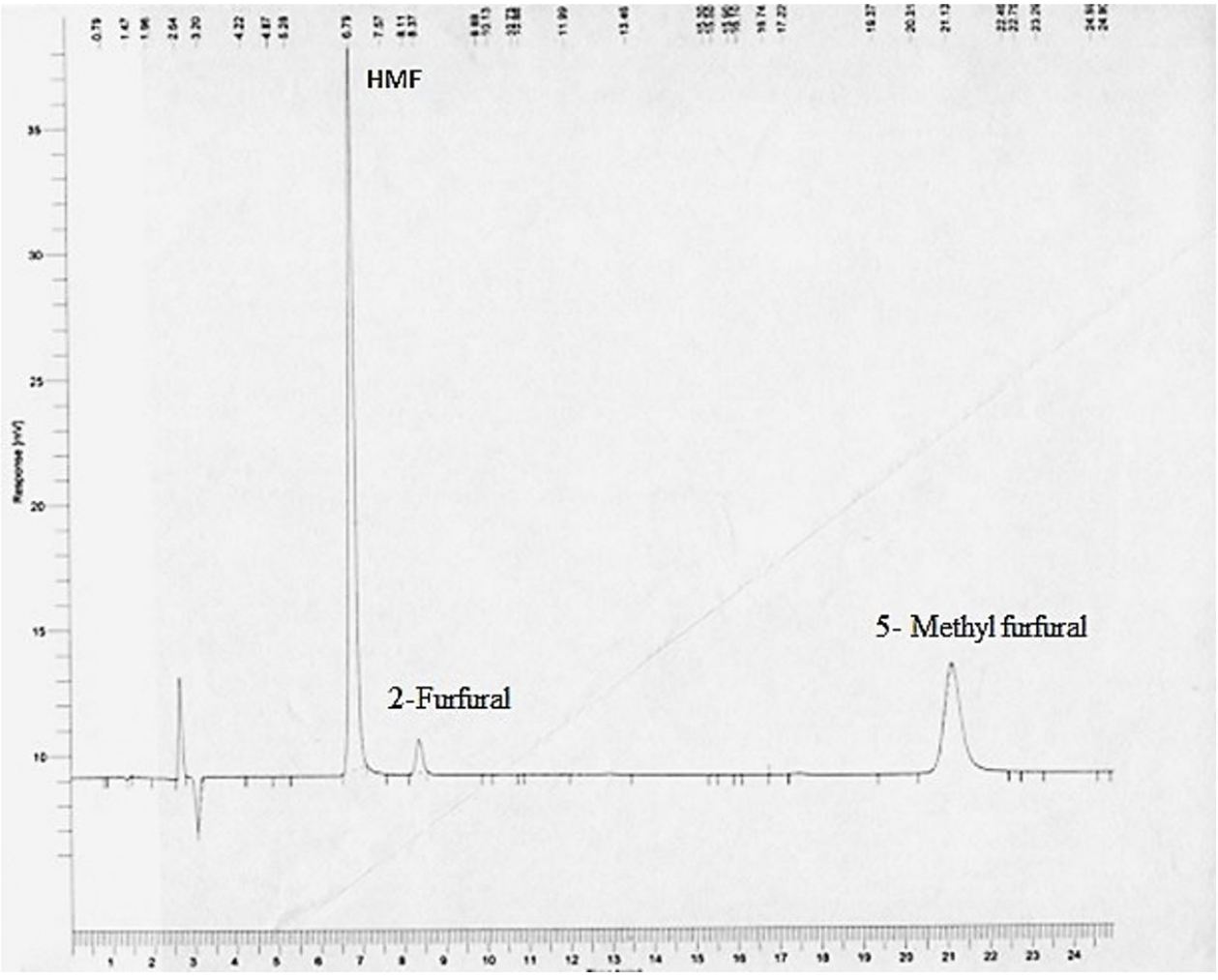

Figure 2. Standard Chromatogram of HMF, 2-Furfural, 5-Methyl furfural.

the storage temperature, as the storage temperature increase the degradation of vitamin C become higher and vice versa. The effect of different storage temperatures on orange juice concentrates was also studied by Burdurlu et al. (2006). They reported that the ascorbic acid content decrease with increasing storage temperature of citrus juice concentrates. They observed that the loss of ascorbic acid was much more in citrus juice concentrates at 45 °C rather than at 28 °C.

Previous literature also exhibited that the ascorbic acid content in different juices decreased during storage, depending on storage conditions, such as temperature, oxygen, and light access (Kabasakalis et al., 2000). Recently, first-order rate constants for nonenzymic browning and ascorbic acid loss of membrane-clarified clear juice, during conventional evaporative processes at 70.3-97.6 °C, have been presented (Johnson et al., 1995).

3.2 Evaluation of furfuraldehydes

The sensitivity of the method for the determination of HMF, 2-Furfural, and 5- Methyl furfural concentrations was checked before using the method. Detection sensitivity of furfuraldehydes was found to be more than any spectrophotometric method. On the basis of 3:1 signal/noise ratio the sensitivity was determined to be <0.015 mg/L for HMF and <0.035 mg/L for both 2-Furfural and 5- Methyl furfural.

HMF concentrations of orange juice are given in the Table 2. After 40 days storage, HMF content of orange juice at 25 °C (T_4) ranged from 0.35 µg/100 mL to 239.01 µg/100 mL. The variation of HMF at 40 °C (T_5) was between 0.35 µg/100 mL to 821.88 µg/100 mL. The increase of HMF at 40 °C was approximately 3.4 times higher than that of at 25 °C. When HMF content in orange juice plotted versus storage time, the best fit model for HMF accumulation was zero-order reaction.

Similarly the concentrations of 2-Furfural was found to be increased during storage and this accumulation was more pronounced due to temperature abuse. The increase in 2-Furfural content of orange juice at 40 °C was approximately 7.4 times higher than that of at 25 °C. In contrast to the above two furfuraldehydes, 5- Methyl furfural which was first time studied with both HMF and 2- Furfural showed a different nature. It showed the maximum accumulation at 25 °C where its concentration was 2.63 µg/100 mL while at 40 °C, a decrease in its concentration was observed.

A significant correlation ranged from 0.675 to 0.880 between vitamin C loss and HMF accumulation ($p < 0.05$) was obtained during the storage of orange juice. Accumulation of HMF in orange juice during storage is mainly attributed by the degradation of ascorbic acid. The same phenomenon was also observed by other scientists in citrus juice concentrates (Burdurlu et al.,

Table 1. Effect of storage temperature and duration on the Ascorbic acid (mg/100 mL) in orange juice.

Temperature (°C)	Storage (Days)				
	0	10	20	30	40
0	34.27 ± 0.21 A	11.67 ± 1.74 B	11.28 ± 2.34 B	10.94 ± 1.79 BC	7.19 ± 1.51 C
5	34.27 ± 0.21 A	4.52 ± 0.32 E	4.14 ± 0.87 E	3.39 ± 1.33 EF	3.04 ± 2.24 F
15	34.27 ± 0.21 A	5.69 ± 1.45 D	3.39 ± 0.54 EF	3.01 ± 0.56 F	2.33 ± 1.39 G
25	34.27 ± 0.21 A	3.39 ± 0.66 EF	3.18 ± 0.8 EF	2.26 ± 0.78 G	2.22 ± 1.67 G
40	34.27 ± 0.21 A	3.05 ± 0.57 F	2.65 ± 0.7 G	2.26 ± 0.45 G	1.89 ± 1.49 H

Letters present in the table showed that data is significantly changing, and values are statistically significant with changing conditions.

Table 2. Effect of storage temperature and duration on the Furfuraldehydes formation (µg/100 mL) in orange juice.

Furfuraldehydes	Temperature (°C)	Storage (Days)				
		0	10	20	30	40
HMF	**0**	0.35 ± 0.07 I	15.57 ± 0.31H	16.18 ± 0.32H	22.38 ± 0.44GH	36.53 ± 0.71G
	5	0.35 ± 0.07I	20.63 ± 0.40GH	25.13 ± 0.49GH	32.28 ± 0.63G	35.4 ± 0.69G
	15	0.35 ± 0.07I	37.82 ± 0.02G	20.99 ± 0.41GH	21.46 ± 0.42GH	25.26 ± 0.49GH
	25	0.35 ± 0.07I	93.58 ± 1.83F	103.47 ± 2.03EF	141.74 ± 2.78E	239.01 ± 4.68D
	40	0.35 ± 0.07I	362.6 ± 7.11C	418.94 ± 8.21 BC	475.21 ± 9.32 B	821.88 ± 16.11A
2- Furfural	**0**	0.85 ± 0.018 J	1.06 ± 0.02 IJ	1.08 ± 0.02 IJ	1.22 ± 0.02HI	1.4 ± 0.02 H
	5	0.85 ± 0.018 J	1.02 ± 0.02IJ	1.12 ± 0.02 I	1.19 ± 0.03 HI	2.28 ± 0.04 G
	15	0.85 ± 0.018 J	2.26 ± 0.05G	2.8 ± 0.06 FG	4.22 ± 0.08EF	15.93 ± 0.32 D
	25	0.85 ± 0.018 J	3 ± 0.06F	4.48 ± 0.09EF	6.87 ± 0.14E	35.28 ± 0.730 C
	40	0.85 ± 0.018 J	2.78 ± 0.06 FG	19.63 ± 0.41D	143.13 ± 2.96 B	260.23 ± 5.38 A
5- Methyl furfural	**0**	ND	ND	0.88 ± 0.03 EF	1.28 ± 0.04 DE	1.94 ± 0.06 B
	5	ND	0.87 ± 0.03 EF	1.02 ± 0.04 E	1.22 ± 0.04 DE	1.49 ± 0.05 CD
	15	ND	1.33 ± 0.05 D	1.47 ± 0.05 CD	1.65 ± 0.06 C	1.94 ± 0.06 B
	25	ND	1.46 ± 0.05 CD	1.8 ± 0.06 BC	1.8 ± 0.06 BC	2.63 ± 0.09 A
	40	ND	ND	ND	ND	0.94 ± 0.03

Letters present in the table showed that data is significantly changing, and values are statistically significant with changing conditions. ND = Not Detected.

2006). However, sugar degradation may also be contributed to HMF accumulation in orange juice to some extent.

Tatum et al. (1969) also conducted a work upon the degradation products from ascorbic acid and identified the following furans: furfural, 2-acetyIfuran, 2, 2-difurylmethane, furfuryl alcohol, 2-hydroxyacetyl furan, 2,5-dihydrofuroic acid, deoxyfuroin, 2-furoic acid, furoin, and furil. They confirmed the identity of most of the compounds by comparing their infrared and ultraviolet spectra, mass spectral cracking patterens and retention times on Carbowax 20M with those of commercially available samples. Nagy & Randall (1973) measured the furfural during the 16 weeks of storage in canned orange juice at 5, 10, 16, 21, and 30 °C. Their studies revealed that at every 5 °C, rise in temperature, there was an approximate double increase in the amount of furfural in canned orange juice. Organoleptic evaluation showed that at the level of 55 µg/L furfural content, the taste of juice was unacceptable to taste panel.

4 Conclusion

It was concluded from above mentioned results that ascorbic acid in orange juice decreased with increasing temperature. The loss of ascorbic acid in orange juice at all storage temperatures was described as a zero-order reaction. On the other hand HMF, 2-Furfural, and 5- Methyl furfural accumulation of orange juice increased depending on storage temperature. It was also observed that the increase of HMF, 2- Furfural and 5- Methyl furfural accumulation in orange juice at 40 °C was higher than that of at 25 °C. An inverse relationship was found between ascorbic acid and furfuraldehyde accumulation. HMF, 2-Furfural and 5- Methyl furfural concentrations of orange juice were found to be increased with an increase in ascorbic acid degradation. Hence, ascorbic acid content was reduced so as vitamin C activity.

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